The Examiner objected to the format of the Specification. Applicants have rewritten the Specification to include the appropriate section headings in the appropriate locations of the Specification.

II. Objections to the Disclosure

The Examiner objected to the following informalities in the Disclosure: (1) the meaning of the phrase "associated to papillomaviruses" on pages 1, 2, 4 and 5; (2) the term "benignant" on page 1; and (3) the phrase "possible for it to integration" on page 2.

Applicants have replaced the phrase "associated to papillomaviruses" with the phrase --infected with-- (page 1, line 24; page 2, line 4 and page 5, line 12 of the "clean" disclosure) or deleted it in its entirety (page 4, line 43 of the marked-up disclosure); replaced "benignant" with --benign-- (page 1, line 32 of the "clean" disclosure); and have replaced the phrase "possible for it the integration" with --facilitate the integration-- (page 2, line 32 of the clean disclosure).

The Examiner objected to the inconsistent use of the term "respectively" throughout the Specification. Applicants have rewritten the substitute Specification wherein "respectively" is properly used as an adverb with the common meaning "in regard to each of two or more, in the order named"[2].

The Examiner objected to an inconsistency relating to the function of the E6 and E7 proteins. Applicants maintain that by nature both E-6 and E-7 have transforming ability. The specification teaches that the early papillomavirus proteins of the E6- and E-7 ORFs are transforming polypeptides (in the sentence bridging pages 1 and 2). It is only through intervention by the deletion of a part of the E6-ORF, that the transforming ability of E6 is destroyed. Similarly, an HPV 16 L1-E7 fusion polypeptide is constructed using a truncated E-7 ORF (page 5, line 40 and 41 of the "clean" specification).

III. Claim Ojections

The Examiner objects to claim 66 under 37 CFR § 1.75(c), as being of improper dependent form. Applicants have amended claim 66 to be in proper dependent form.

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[2] Webster's New Word Dictionary 636 (Second Concise Edition 1982)

IV. Claim Rejections – 35 U.S.C. § 112 (First Paragraph)

The applicants' claims satisfy the written description requirement of the first paragraph of 35 U.S.C. § 112. The Examiner objected to claims 62-64 under § 112. The Examiner also stated that the specification, while being enabling for a vaccine composition comprising the adeno-associated virus vector as set forth in claim 14 and an auxiliary agent, does not reasonably provide enablement for a vaccine composition wherein the vector is provided as a component of a cell, wherein the cell is a tumor or pre-tumor cell with human papillomavirus infection, and wherein the cell is inactivated. Further, the Examiner states that the specification does not disclose a vaccine composition wherein the vector is provided as a component of a cell, wherein the cell is a tumor or pre-tumor cell and is associated with human papillomavirus infection, and wherein the cell is inactivated.

The specification teaches a vaccine composition comprising a cell transfected with the vector of claim 14. The Examiner's attention is respectfully directed to page 5, lines 2 through 5 of the specification wherein applicants state, "It is particularly favorable for the vaccination agent to also contain the cells transduced by the vector". The various auxiliary substances referred to earlier in the same paragraph are applied to cell vaccines by the next statement that concludes with the phrase "as the above explanations apply to the cells as well". In addition, applicants state in the last sentence of said paragraph, "If tumor or pre-tumor cells are used, it will be favorable for the cells to be inactivated".

Still further, the Examiner's attention is respectfully directed to page 2, lines 38-40 of the substitute specification, wherein applicants define "papillomavirus" as comprising "any papillomavirus or fragments thereof, which can be found in host cells, particularly tumor cells". Accordingly, applicants have disclosed in the specification a vaccine composition wherein the vector may be provided as a cell component, wherein the cell is a tumor or pre-tumor cell and is associated with human papillomavirus infection, and wherein the cell is inactivated. Applicants provide guidance with respect to inactivation, by recommending irradiation, a commonly used method of inactivating cells.

The Examiner states that the specification does not provide any guidance as to how to make or use a vaccine composition wherein the vector is provided as a component of a cell. Tumor-cell

vaccines are well known to those of skill in the art. Accordingly, one of skill in the art would readily understand that the applicants were at time of filing in possession of a tumor-cell vaccine based on the fusion polypeptide encompassed by the pending claims.

V. Claim Rejection – 35 U.S.C. § 112 (Second Paragraph)

The applicants' claims satisfy the written description requirement of the second paragraph of 35 U.S.C. § 112. The Examiner has rejected claims 14-48, 51, 52 and 60-66 as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention under the second paragraph 35 U.S.C. §112. However, the Examiner has conceded the enablement of the invention for specifically exemplified aspects of the invention, viz.:

> an AAV vector comprising a nucleic acid coding for a fusion polypeptide comprising a structural papillomavirus polypeptide and anon-transforming polypeptide, wherein the fusion polypeptides comprise a polypeptide coded by HPV-16 L1-ORF and a polypeptide coded by HPV 16 E6-ORF, and another fusion polypeptide coded by HPV 18 L1-ORF and a polypeptide coded by HPV 16 E6-ORF and E7-ORF (Office Action of February 2, 2000, paragraph bridging pages 4 and 5).

Amended claims are directed to such combinations as those, conceded by the Examiner to be enabled. In addition to the exemplified aspects of the invention, applicants' disclosure enables one skilled in the art to obtain, without undue experimentation, more than what is discussed by the Examiner in the above paragraph. One skilled in the art can readily obtain fusion peptides as recited in the amended claims, wherein (1) the structural papillomavirus polypeptide is encoded by L1-ORF, L2-ORF or fragments thereof, and (2) the early papillomavirus polypeptide is encoded by E1-ORF, E2-ORF, E4-ORF, E5-ORF, E6-ORF, E7-ORF, or fragments thereof, wherein said early papillomavirus peptide is non-transforming. It must be emphasized that assertions of indefiniteness under § 112, second paragraph, are considered from the point of view of those skilled in the art. Morton Int'l, Inc. v. Cardinal Chemical Co., 5F.3d 1464, 1470 (Fed. Cir. 1993). A claim is not invalid for indefiniteness under 35 U.S.C. § 112, second paragraph, unless one of skill in the art would not understand what is claimed when read in light of the specification.

The Examiner has also characterized applicants' claims 14, 26, 28, 31, 33, 35, 37, 39, 41, 43-48, 51, 52 and 65 as vague and indefinite by the term "fragment(s)". However, in view of applicants'

disclosure and without undue experimentation, one skilled in the art can readily obtain fusion polypeptides as recited in the claims.

Applicants concede that some experimentation will be necessary to define the functional limitations of any such fragment(s) but it is well settled that the enablement requirement permits some experimentation, so long as that experimentation is not undue.

In PPG Industries, Inc. v. Guardian Industries, Corp., the court stated that even where some experimentation is necessary to reduce an invention to practice, the enablement requirement is satisfied where: (1) the experimentation is routine; or (2) the specification provides "a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed" (PPG Indus., Inc. v. Guardian Indus. Corp., 37 U.S.P.Q.2d 1618, 1623 (Fed. Cir 1996)).

Applicants point out that guidance is provided in the disclosure wherein fragments of L1-ORF and E7-ORF are used to create a fusion gene. Through routine test protocols, one skilled in the art will be able to test the efficacy of the vectors in stimulating the immune system against PVs and their host cells. Further, the claims are limited by the amino acid chain lengths of the unfragmented (poly)peptides which are well defined in the literature.

Applicants have rewritten claim 14 using proper Markush language.

Applicants have rewritten claims 61, 62 and 63.

The term inactivated in claim 64 refers to the destruction of the tumorigenic activity of the subject tumor or pre-tumor cells. The claim has been rewritten removing the phrase in question "wherein the cell is inactivated". It is well known to one of ordinary skill in the art that the transforming or tumorigenic activity of cells amy be destroyed through irradiation of the subject cells.

Accordingly, all claims are enabled, and the applicants respectfully request that the Examiner withdraw the enablement rejections under the second paragraph of 35 U.S.C. § 112.

VI. Claim Rejection – 35 U.S.C. § 103

The pending claims are non-obvious in view of Donnelly et al. (WO 96/00583) taken with Johnson (U.S. Patent 5,658,785).

Amended claim 14 (which corresponds to original claim (1)) requires the following components:

(a) a structural papillomavirus polypeptide encoded by L1-ORF, L2-ORF, or fragments thereof; and

(b) an early papillomavirus polypeptide encoded by E1-ORF, E2-ORF, E4-ORF, E5-ORF, E6-ORF, E7-ORF, or fragments thereof, wherein said early papillomavirus polypeptide or fragment thereof is non-transforming.

The Examiner contends that: (1) Donnelly et al. teaches vaccines comprising DNA constructs encoding papillomavirus gene products that are capable of being expressed upon direct introduction into animal tissues, and prophylactic and therapeutic pharmaceuticals that can provide immune protection against infection by papillomavirus; (2) Donnelly et al. does not teach an adeno-associated virus (AAV) vector comprising the papillomavirus polynucleotides encoding fusion polypeptides, but that (3) Johnson teaches AAV vectors comprising a constitutive or inducible promoter which are suitable for delivering foreign DNA to cells, including that obtained from pathogens and which can be used for genetic immunization.

Applicants agree that such a fusion polypeptide would satisfy the requirements of (a) above for a structural polypeptide, but there is no teaching or suggestion of a fusion polypeptide with the components of a structural polypeptide in combination with a non-transforming element. Further, a serious question of safety would surround the use of a transforming element (e.g., polypeptide coded by a full length E7-ORF) in an AAV vector. However, the applicants solve this problem by truncating the E7-ORF to destroy its transforming ability. The use of a non-transforming element as part of a fusion construct is not taught by the cited references; therefore, the cited references do not render the claimed invention obvious.

VII. Claim Rejection – 35 U.S.C. § 103

The pending claims are non-obvious in view of Donnelly et al. (WO 96/00583), Johnson (U.S. Patent No. 5,658,785) and Whittle et al. (U.S. Patent No. 5,955,087).

The Examiner rejects claims 14-60, 65, and 66 under 35 U.S.C. 103 (a) as being unpatentable over Donnelly et al., taken with Johnson, as applied to claims 4-25, 27-30, 32, 34, 36, 38, 40, 42-60, 65, and 66 above, and further in view of Whittle et al. This rejection is applied to the claims directed to adeno-associated virus vectors comprising polynucleotides encoding HPV fusion proteins, wherein the fusion proteins comprise fragments of the (poly)peptides of (a) and (b).

Applicants agree that such a fusion polypeptide would satisfy the requirements of (a) above for a structural polypeptide for the production of neutralizing antibodies. However, there is no teaching in any of the references cited including Whittle that is directed to modification or fragments of early papillomavirus peptides to effect a non-transforming element. In the present invention, fragments of the (poly)peptides of (b) are selected with respect to their non-transforming ability as compared to full-length (poly)peptides and not with respect to their antigenic determinants. Applicants utilize a fragment of E7-ORF in their preparation of a vector coding for an HPV 16 L1-E7 fusion polypeptide.

The use of a non-transforming fragment of an early papillomavirus polypeptide as part of a fusion construct is not taught by the cited references; therefore, the cited references do not render the claimed invention obvious.

VIII. Claim Rejection – 35 U.S.C. § 103

The pending claims are non-obvious in view of Donnelly et al. (WO 96/00583), Johnson (U.S. Patent No. 5,658,785) and Gissmann et al. (WO 96/11272).

The Examiner rejects claims 16, 18, 20 and 50 under 35 U.S.C. § 103 (a) as being unpatentable over Donnelly et al., taken with Johnson as applied to claims 14-25, 27-30, 32, 34, 36, 38, 40, 42-60, 65, and 66 above, and further in view of Gissmann et al. The Examiner's rejection applies to the claims directed to adeno-associated virus vectors comprising polynucleotides encoding HPV fusion proteins, wherein the fusion proteins are full length proteins or fragments thereof obtained

from the L1, L2, E1, E2, E4, E5, E6 and E7 human papillomavirus proteins and wherein the HPV types include HPV 33, HPV 35 and HPV 45.

None of the above references teach or suggest the use of a non-transforming early papillomavirus as part of a fusion construct. Further, the use of a fusion construct wherein the early papillomavirus polypeptide is not modified to render it non-transforming may present safety issues.

The use of a non-transforming early papillomavirus polypeptide as part of a fusion construct is not taught by the cited references; therefore, the cited references do not render the claimed invention obvious.

IX. Claim Rejection – 35 U.S.C. § 103

The pending claims are non-obvious in view of Donnelly et al. (WO 96/00583), Johnson (U.S. Patent No. 5,658,785) and Stanley et al. (U.S. Patent 6,096,869).

The Examiner rejects claim 61 under 35 U.S.C. 103(a) as being unpatentable over Donnelly et al., taken with Johnson as applied to claims 14-25, 27-30, 32, 34, 36, 38, 40, 42-60, 65, and 66 above, and further in view of Stanley et al. which teaches a pharmaceutical treatment material comprising a vaccine adjuvant (i.e., an immune system activating agent) and a vector encoding and able to cause expression of a papillomavirus protein or antigenic fragment or fusion protein corresponding thereto.

None of the above references teach or suggest the use of a non-transforming early papillomavirus as part of a fusion construct. Further, the use of a fusion construct wherein the early papillomavirus polypeptide has transforming ability may present safety issues.

The use of a non-transforming early papillomavirus polypeptide as part of a fusion construct is not taught by the cited references; therefore, the cited references do not render the claimed invention obvious.

The various § 103 rejections of the pending claims are based on citation of various references that are wholly devoid of any teaching or suggestion of AAV vectors, and such reference therefore

provide no basis or motivation for combination with a reference mentioning AAV (viz., Johnson et al.) but containing no teaching or suggestion of HPV vaccination. The various reference combinations, the deficiencies of which have been amply discussed hereinabove in relation to the pending claims, find basis only when using hindsight in an attempt to reconstruct applicants' invention. Accordingly, such hypothetical aggregation of references provides no derivative basis for applicants' claimed invention.

Further, it is to be noted that the combination of an AAV vector and a papillomavirus fusion protein yields surprising and highly unexpected properties. The AAV vector enables an intracellular expression of the fusion gene (e.g., L1-E7 of HPV 16) and presentation of the antigen via an MHC-I mode (therapeutic immunization). Additionally, the fusion gene itself allows intracellular production of HPV 16 "virus-like particles" capable of leaving the cell and infecting antigen-presenting cells. Such action results in presentation of the antigen via an MHC-II mode and thereby to a neutralization of the HPV infection (prophylactic immunization). This "double effect" could not be foreseen from any combination of the references of record, and underscores the non-obviousness of applicants' claimed invention.

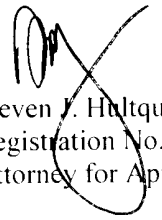
CONCLUSION

In view of the foregoing amendments and remarks, it is clear that claims 14-66 now pending in the application comply with 35 U.S.C. § 112, embody novel and non-obvious subject matter, and are in form and condition for allowance.

The Examiner is respectfully requested to take cognizance of the claims as amended herein, and to responsively issue a Notice of Allowability and Notice of Allowance for claims 14-66.

In the event that any issues remain outstanding, incident to the formal allowance of the application, the Examiner is requested to contact the undersigned attorney at (919) 419-9350 to discuss their resolution, in order that this application may be passed to issue at an early date.

Respectfully submitted,



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APPENDIX C

TITLE OF THE INVENTION

Vector for Activating the Immune System Against Cells Infected with Papillomaviruses, Papillomaviruses or Fragments Thereof.

CROSS REFERENCE TO RELATED APPLICATIONS

This application was filed under 35 U.S.C. § 371, which was the National Stage of International Application No. PCT/DE97/01629, filed 07/30/97.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to an adeno-associated virus (AAV) vector comprising a nucleotide sequence that encodes a fusion polypeptide comprising a structural papillomavirus polypeptide and an early papillomavirus polypeptide or fragments thereof, respectively. This vector may be used to activate the immune system against papillomavirus particles or fragments thereof, and/or cells infected with papillomaviruses, particularly tumor cells transformed by papilloma virus infection. This vector may be used as a therapeutic treatment or as a part of a vaccine protocol.

2. Description of Related Art

Papillomaviruses (PVs) infect the epithelial cells of a wide range of animals, including humans. Human papillomaviruses (HPVs) are the cause of benign and malignant growths. HPVs have been found for example in benign warts, condylomas in the genital region, and in malignant

carcinomas of the skin and uterus (e.g., epithelial neoplasms). HPVs may also be responsible for the development of malignant tumors in the respiratory system including squamous cell carcinomas.

Papillomaviruses have an icosahedral capsid that surrounds a circular double-stranded DNA molecule of about 7900 base pairs. The capsid comprises a major capsid protein (L1) and a minor, capsid protein (L2) but lacks an envelop. The former capsid protein is coded by open reading frame L1 (L1-ORF) and the latter is coded by open reading frame L2 (L2-ORF). *In vitro* expression of L1 or L1 and L2 results in the formation of virus-like particles (VLPs). Further, the ability of PVs to transform infected or host cells is ascribed to the proteins E6 and E7 which are coded by E6- and E7-ORFs, respectively.

Many attempts have been made to stimulate the immune system over cells already infected with PVs (host cells), PVs and fragments thereof. However, these attempts have not yet yielded satisfactory results.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a vector having a nucleic acid coding a fusion polypeptide, the fusion polypeptide comprising a structural papillomavirus (poly)peptide and a non-transforming (poly)peptide coded by an early papillomavirus gene. This fusion polypeptide provides a means for activating the immune system to identify and eliminate host cells infected with PVs, PVs and fragments thereof, and in particular those host cells which have been transformed to a tumorigenic phenotype.

As used herein, "vector" comprises any vector which is suitable for gene transfer, for example, those capable of introducing nucleic acids into cells. A vector may remain episomally or be

integrated within the genome of a host cell. Moreover, the vector may be a plasmid or virus vector. The genomes of retroviruses, adenoviruses, vacciniaviruses and adeno-associated viruses (AAV), the latter being preferred, have been adapted as highly efficient vectors for introducing genes into cells. An AAV vector may be present in wild-type or modified form. In modified form, it may comprise two inverted terminal sequences (ITRs), that are required for its transduction ability. However, depending on the application, it may also be advantageous for such a modified AAAV vector to comprise additional sequences, such as Rep sequences, which facilitate the integration of nucleic acids (genes) into chromosome 19. A virus vector can be present as a viral particle or in the form of its nucleic acid. It is preferred for the virus vector to be replication-defective.

As used herein, "papillomavirus" comprises any papillomavirus or fragments thereof, which can be found in host cells, particularly tumor cells. In particular, HPVs and more particularly "high risk" HPVs, such HPV 16, 18, 33, 35 and 45.

As used herein, "nucleic acid" comprises any nucleic acid such as DNA and/or RNA, which codes for a fusion polypeptide comprising a structural papillomavirus (poly)peptide and a non-transforming (poly)peptide coded by an early papillomavirus gene. It is favorable for the nucleic acid to be expressible. It is particularly favorable for it to be controlled by a constitutive or inducible promoter such as a tissue-specific or tumor-specific promoter.

As used herein, "structural papillomavirus (poly)peptide" comprises any peptide or polypeptide of a papillomavirus, which is at least responsible in part for the structure of the papillomavirus. In particular, such a (poly)peptide is coded by L1-ORF or L2-ORF, or fragment thereof of a papillomavirus. A (poly)peptide which can be present as VLP is particularly preferred.

As used herein, "transformation" refers to the conversion of a normal cell into a tumor cell which has the capacity for unlimited proliferation.

As used herein, "a non-transforming (poly)peptide" comprises any peptide or polypeptide, that is coded by an early papillomavirus gene (ORF) or fragment thereof, and is non-transforming by nature or through intervention. The early papillomavirus genes include but are not limited to the E1-, E2-, E4-, E5-, E6- and E7-ORFs. Through intervention, the transforming ability of a (poly)peptide is destroyed by deleting a part of the ORF. A preferred non-transforming (poly)peptide is coded by a fragment of the E6- or E7-ORFs of a papillomavirus.

As used herein, "fusion polypeptide" refers to the fact that the structural papillomavirus (poly)peptide and the non-transforming (poly)peptide coded by an early papillomavirus gene can be present in any combination with the fusion polypeptide. The individual (poly)peptides may also originate from different papillomaviruses. The C terminus of the structural (poly) peptides is preferably connected with the N terminus of the non-transforming (poly)peptide. In addition, it may be advantageous for the non-transforming (poly)peptide to be localized within the structural (poly)peptide. A preferred fusion polypeptide comprises a (poly)peptide coded by HPV 16 L1-ORF and a (poly)peptide coded by HPV 16 E6-ORF or E7-ORF, respectively. Furthermore, a fusion polypeptide is preferred which comprises a (poly)peptide coded by HPV 18 L1-ORF and a (poly)peptide coded by HPV 18 E6-ORF or E7-ORF, respectively.

Common methods can be carried out for the preparation of above vectors. For example, an AAV vector can be prepared as a virus particle as follows: the 5' end of the HPV 16 E6-ORF is ligated to the 3' end of the HPV 16 L1-ORF. Part of the E6-ORF has been deleted beforehand, so that the transforming properties of E6 were destroyed. The DNA fragment L1-ORF-E6-ORF is inserted in a modified AAV vector which contains the 5' –ITR and 3' ITR sequences of AAV but not the

sequences coding for the AAV Rep and AAV Cap proteins. The insertion is made between the two ITR sequences. The DNA fragment L1-ORF-E6-ORF is controlled by a promoter heterologous with respect to AAV. The resulting AAV vector is transfected into cells, which express the AAV Rep and AAV-Cap proteins. Furthermore, the cells are infected with a helper virus, e.g. adenovirus, so that the AAV vector is obtained as a viral particle.

The immune system can be activated with the above vector, to identify and eliminate host cells, particularly tumor cells, transformed by papillomaviruses and or PVs and fragments thereof, respectively. This can be achieved prophylactically or as a therapeutic treatment. For this purpose, cells of the particular organism, such as antigen-presenting cells, e.g. dendritic cells, B cells, macrophages and/or tumor cells and/or pre-tumor cells are transduced with the vector. The transduction can be made by common methods. If the vector is available as a virus particle, it will be favorable to infect the cells therewith. On the other hand, if it is available as a nucleic acid, e.g. DNA, it will be advisable to transfect the cells therewith. Electroporation, lipofection and particle gun have to be mentioned as transfection techniques by way of example. The cells may be present in the organism. On the other hand, the cells to be transduced can also be isolated from the organism, transduced outside the organism and then returned to the organism again. Such cells are referred to as autologous cells. Moreover, allogenic cells can also be used for the transduction regarding the organism. In this connection, it is favorable for these cells to belong to an HLA type corresponding to the organism. The person skilled in the art is familiar with processes of providing cells with a certain HLA type. In addition, it is favorable if, in an above process, the tumor cells or pre-tumor cells are inactivated before they are returned to the organism. For this purpose, common methods, such as irradiation, can be used.

Another subject matter of the present invention relates to a vaccination agent which comprises an above vector and common auxiliary substances, such as buffers, diluents, carriers, etc.. It can be advantageous for the vaccination agent to contain additional substances which can activate the

immune system, e.g. against tumor cells. Such substances include but are not limited to MHC-1 molecules, co-stimulatory molecules, e.g. B7, and secretory immunostimulators, for example, cytokines, such as IL-2, IL-12, interferon and GM-CSF. These substances can be present for example, in the form of peptides, particularly synthetic peptides.

The substances can also be present in the form of expression plasmids encoding them, which can also code for HLA molecules. It is particularly favorable for the vaccination agent to also contain the cells transduced by the vector. Host cells, particularly tumor or pre-tumor cells may be isolated from the organism, transduced by a variety of methods and returned to the organism as a therapeutic treatment as the above explanations apply to the cells as well. If tumor or pre-tumor cells are used, it will be favorable for the cells to be inactivated.

By means of the present invention it is possible to activate the immune system against the host cells infected with papillomaviruses, PVs and or fragments thereof, respectively. These cells include tumor cells and pre-tumor cells, respectively. The activation of the immune system can be made prophylactically or as a therapeutic treatment. The present invention represents a new step of treating the most severe diseases via in *in vivo* gene therapy and *ex vivo* gene therapy, respectively.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENT

The present invention is illustrated by the following example relating to the production of viral particles which incorporate the subject fusion polypeptide.

Example: Preparation of a Vector Coding for an HPV16 L1-E7 Fusion Polypeptide

The L1-ORF of a genomic HPV16 clone (Kirnbauer et al. (1993)J. Virol. 12: 6929-6936) was amplified by PCR reaction. For this purpose, L1-specific primers were used which have an additional Bg1III restriction site at the 5' end. The amplified DNA fragment was cleaved using Bg1III and inserted in BamHI restriction site of the common vector pUC19. An EcoRV restriction site, followed by a translation stop codon (TAA), was introduced at position 7051 of the L1-ORF by specific mutagenesis. By this, it was achieved that the L1-ORF coded for an L1 which was lacking the last 34 amino acids.

In another PCR reaction, the part of the E7-ORF of HPV16 was amplified which codes for the first 50 amino acids of E7. The employed primers included an EcoRV restriction site at their 5' end. The amplified DNA fragment was inserted in the EcoRV restriction site of the above pUC19 vector which codes for the shortened L1. Thus, an L1-E7 fusion gene was obtained. It was inserted in the common baculovirus vector pVL1392 via XbaI/SmaI. The L1-E7 fusion gene was cleaved therefrom by NotI/SmaI and inserted in the NotI restriction site of the AAV vector pUF2 (Zolotukhin et al., J. Virol. 70, (1996), 4646-4654). A vector was obtained which codes for an HPV16 L1-E7 fusion polypeptide. Viral particles of the vector were obtained according to common methods (Rolling and Samulski (1995) Molecular Biotechnology 3: 9-15).